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Whole-genome sequences of Cronobacter sakazakii isolates obtained from plant-origin foods and dried food manufacturing environments

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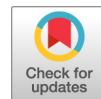


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Whole-Genome Sequences of *Cronobacter sakazakii* Isolates Obtained from Foods of Plant Origin and Dried-Food Manufacturing Environments

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ABSTRACT Here, we present draft genome sequences of 29 *Cronobacter sakazakii* isolates obtained from foods of plant origin and dried-food manufacturing facilities. Assemblies and annotations resulted in genome sizes ranging from 4.3 to 4.5 Mb and 3,977 to 4,256 gene-coding sequences with G+C contents of ~57.0%.

Cronobacter species are Gram-negative opportunistic pathogens associated with life-threatening infections, such as infantile meningitis, septicemia, and necrotizing enterocolitis (1, 2), and urinary tract infections, pneumonia, and wound infections in adults (3, 4). The *Cronobacter* genus consists of seven species, including *C. sakazakii*, *C. malonicus*, *C. turicensis*, *C. muytjensii*, *C. dublinensis*, *C. universalis*, and *C. condimenti* (5, 6). The primary pathogen is *C. sakazakii*, and epidemiologically, infections have been linked to consumption of contaminated powdered infant formula. However, surveillance studies have shown that *Cronobacter* spp. are found in a variety of different foods, including dried foods (flour, spices, herbs, and cereal) and fresh ready-to-eat vegetables (7–10). There is a growing body of evidence that plants may serve as a reservoir or ancestral host for *Cronobacter* spp. (11, 12). Although occurrences of *Cronobacter* spp. in foods of plant origin are increasingly being reported, relatively little genomic information on them is available. Here, we report the draft genome sequences of 29 *C. sakazakii* strains isolated from foods of plant origin and dried-food processing environments.

Whole-genome sequencing (WGS) libraries of these *C. sakazakii* strains were constructed using the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA, USA). FASTQ data sets generated on the MiSeq platform (Illumina) were trimmed for *de novo* assembly using CLC Genomics Workbench version 9.0 (CLC bio, Aarhus, Denmark). The genomes were annotated using the Rapid Annotations using Subsystems Technology (RAST) annotation server (13), and 16 sequence types were determined by using the *Cronobacter* multilocus sequence typing (MLST) website (<http://pubmlst.org/cronobacter>). The genome sizes and coding sequences (CDSs) of these assemblies ranged from 4.3 to 4.5 Mb and 3,977 to 4,256, respectively, with G+C contents of ~57.0% (Table 1).

All strains harbored a pESA3/pSP291-like virulence plasmid, which was found by comparing their genome assemblies with WGS of *C. sakazakii* BAA-894 (GenBank accession numbers NC_009778 and CP000783) and was confirmed by PCR analysis (14). Four strains also possessed pESA2, and three possessed pCTU3.

Other mobilome-like genes, such as integrase/transposase genes coding for COG0582, Tn7 (TnsA), and TnpA, and 19 to 40 phage-associated proteins, were present in some strains. Interestingly, all strains possessed a fosfomycin resistance (*fosA*) gene. Other genes identified in these strains included genes for multidrug resistance efflux

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TABLE 1 Genomic information and GenBank accession numbers of 29 *C. sakazakii* isolates obtained from foods of plant origin and dried-food production environments

Strain	Genome size (kb)	G+C content (%)	No. of CDSs	ST ^a	Source	GenBank accession no.
MOD1_LR631	4,485	56.8	4,134	4, CC4 ^b	Instant oatmeal	PTPF000000000
MOD1_LR632	4,552	56.7	4,225	4, CC4	Environment	PTPE000000000
MOD1_LR634	4,421	56.9	4,118	148, CC16	Dried cut carrots	PTPD000000000
MOD1_LR635	4,428	56.9	4,096	148, CC16	Dried cut carrots	PTPC000000000
MOD1_LR640	4,553	56.7	4,231	1, CC1	Sodium caseinate	PTPB000000000
MOD1_LR654	4,433	57.0	4,061	23, CC23	Adult cereal	PTPA000000000
MOD1_LR707	4,406	57.0	4,055	1, CC1	Org. ^c casein flour	PTOZ000000000
MOD1_LR708	4,441	56.9	4,106	1, CC1	Org. casein flour	PTOY000000000
MOD1_LR715	4,503	56.9	4,179	4, CC4	Org. soy	PTOW000000000
MOD1_LR722	4,489	56.7	4,147	1, CC1	Org. soy	PTOV000000000
MOD1_LR733	4,468	56.9	4,142	1, CC1	Org. casein flour	PTOU000000000
MOD1_LR752	4,363	56.9	4,034	1, CC1	Honey powder	PTOT000000000
MOD1_LR753	4,545	56.7	4,268	31, CC31	Honey powder	PTOS000000000
MOD1_LR757	4,576	56.8	4,256	13, CC13	Org. casein flour	PTOR000000000
MOD1_1-15	4,459	56.9	4,086	136	Nuts	NITJ000000000
MOD1_3-21	4,420	56.9	4,069	1, CC1	Nuts	NITK000000000
MOD1_5-17G	4,422	56.9	4,071	226, CC8	Nuts	NITM000000000
MOD1_5-20G	4,567	56.7	4,215	17, CC17	Nuts	NITL000000000
MOD1_5-21G	4,569	56.8	4,230	17, CC17	Nuts	NIXM000000000
MOD1_KW1	4,539	56.8	4,155	93	Barley	NITI000000000
MOD1_KW4	4,542	56.8	4,233	73, CC73	Dried seaweed	NITG000000000
MOD1_KW11	4,446	56.8	4,085	6 alleles ^d	Black bean	NITE000000000
MOD1_KW18	4,438	57.0	4,095	156, CC21	Mushroom	NITC000000000
MOD1_777122	4,375	57.0	4,057	198, CC52	Hulled sesame seed	PTOQ000000000
MOD1_760029	4,508	56.8	4,197	40, CC40	Whole grain, corn	PTOP000000000
MOD1_16MP002184	4,564	56.6	4,238	40, CC40	Chocolate org. shake	PTOO000000000
MOD1_16MP002185	4,508	56.8	4,168	3, CC3	Chocolate org. shake	PTON000000000
MOD1_Jor109	4,412	57.0	3,997	6 alleles ^e	Grapes	NITQ000000000
MOD1_WNTSBCO4	4,490	56.8	4,137	1, CC1	Walnut	PTOM000000000

^aSequence type (ST) was determined by uploading genome assemblies to <https://pubmlst.org/cronobacter>.

^bCC, clonal complex.

^cOrg., organic.

^dThe MLST scheme for strain MOD1_KW11 matched only 6 of the 7 alleles, and no closest match was determined.

^eThe MLST scheme for strain MOD1_Jor109 matched only 6 of the 7 alleles, and its closest match was determined to be ST40, CC40.

pump-related proteins belonging to the *acrAB* operon, the resistance-nodulation-division, the major facilitator superfamily, ABC-type drug transport, and bicyclomycin resistance families. Heavy metal resistance genes and gene clusters involved in copper, hydroperoxide, fusaric acid, and tellurite resistance were also found. An albicidin (a phytotoxin that inhibits DNA gyrase in chloroplasts) resistance protein (11, 15) was observed in all the strains. Furthermore, all strains possessed an operon encoding a xylose utilization pathway, supporting the hypothesis that plants may be the ancestral ecomiche for *Cronobacter* spp., as posited by Chase et al. (11) and Schmid et al. (12). However, the size of this gene cluster varied among the strains.

These results add to the growing number of genomes of *Cronobacter* strains which have plant origins. The availability of genomic information from these strains will provide a better understanding of the genetic features linked to plant association and expands insights into the evolutionary history of this important food-borne pathogen.

Accession number(s). The *C. sakazakii* genome sequences were submitted to NCBI GenBank under BioProject number PRJNA258403 (*Cronobacter* GenomeTrakr Project, FDA-CFSAN), and their accession numbers are listed in Table 1.

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